Frontiers in Structural Biology at High-Brightness X-Ray Sources Workshop, May 21, 2001

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The "Frontiers in Structural Biology at High-Brightness X-ray Sources" Workshop held at Brookhaven National Laboratory on May 21st focused on (1) evolving X-ray structural methods in biology (single particles, monolayers, macromolecular folding, and other time-dependent phenomena) that depend on current high-brightness synchrotron sources, and that may be expected to undergo even greater advances with future potential higher-brightness, pulsed X-ray sources, such as hard X-ray Free Electron Lasers (FELs) or Photoinjected Energy Recovery Linacs (PERLs), and (2) new or recent structures of some extremely important membrane proteins by current X-ray methods, which typically require synchrotron beamlines for satisfactory structure solution, and which are a class of proteins that are notoriously difficult for overall structure determination by any method.

The morning session, chaired by Prof. Caroline Kisker of the State University of New York at Stony Brook (SUNY/SB), presented several structures for which, only a decade ago, it was almost unforeseeable that structures of such biological importance would be solved this early in the new century. The first speaker of the morning was Dr. Peter Orth, who spoke on the crystal structure of the Photosystem-II reaction center (PS-II), based upon work done in the laboratory of Prof. Wolfram Saenger of the Freie Universität in Berlin. This structure represents the Holy Grail for many in photosynthesis, as PS-II is the site of water splitting and oxygen evolution, which generates molecular oxygen on Earth, and is of potential importance for design of solar energy devices. The complex that was crystallized consisted of at least 17 subunits. The structure was determined for a non-crystallographic dimer to a resolution of 3.8 Å; at this resolution, not all of the subunits could be assigned, but C-α carbons could be modeled for almost 2,500 residues, and many chromophores could be assigned. Some of the most exciting results were finding that the 4 manganese atoms that form the catalytic site for water oxidation and oxygen evolution re-

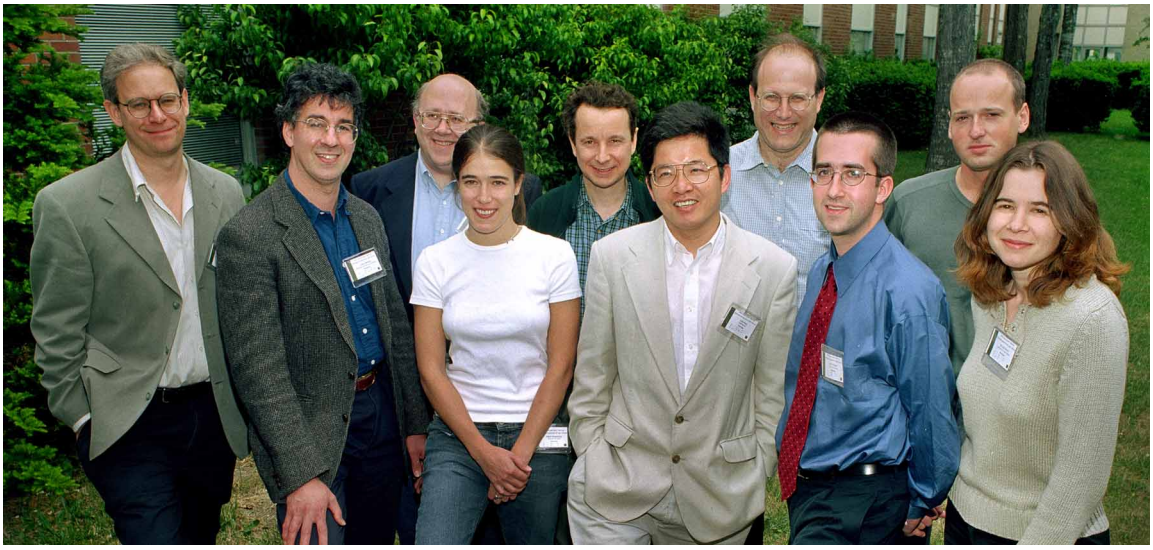
semble a triangle-and-one arrangement, which was not expected from many models based on analysis of EXAFS and other data. This structure will facilitate formulation and testing of advanced models for catalysis of water oxidation and oxygen evolution.

The next speaker was Dr. Katjuša Brejc, who, working in the laboratory of Prof. Titia Sixma of the Netherlands Cancer Institute, determined the structure of a water-soluble complex of Acetylcholine Binding Protein. The structure is highly homologous to the ligand-binding domain of the important family of pentameric ligand-gated ion channels, which has members such as the nicotinic Acetylcholine Receptor. The structure solution was a challenge, requiring 20-fold averaging and density modification using data from 3 different crystal forms. The structure is a homopentamer, where each monomer has a modified immunoglobulin topology. Disulfide bonds of potential functional significance were recognized. One of the particularly intriguing aspects of the work was that it revealed how the ligand-binding pocket is located at the interface of 2 subunits, where each subunit provide different types of interactions for ligand binding. The structure may prove very useful for structure-based design of drugs to treat, for example, Alzheimer's disease and nicotine addiction.

Prof. Rod MacKinnon of the Rockefeller University was the next speaker. Extending his previous impressive work on structure determination of the K^+ channel, he presented recent work on using Rb^+ to investigate electron-density changes in the pore of the K^+ channel. This allowed him to suggest a detailed molecular model for ion conductance through the channel. He also presented a structure of the K^+ channel at higher resolution than previously determined; this was made possible by crystallizing a complex of the channel with F_{AB}s directed against it, and by collecting data at beamline X25 of the NSLS. The structure provided a beautiful high-resolution view of K^+ ions in the pore of the channel, which is lined with peptide backbone carbonyl groups.

Prof. Ron Stenkamp of the University of Washington in Seattle concluded the morning session with the structure of bovine rhodopsin, which represents the first structure of the biologically critical class of G-protein-Coupled Receptors. Ron cited the heroic efforts of Tetsuji Okada, who purified protein from bovine rod outer segments, and who found that Zn^{2+} was a key ingredient necessary to yield well-diffracting crystals. Although the crystals were typically twinned, the structure could be determined to 2.8-Å resolution by multi-wavelength anomalous diffraction phasing of a mercury derivative. (Perhaps this qualifies as a MAD cow experiment?) As expected, the overall fold consisted of 7 transmembrane helices, but the structure revealed why bacteriorhodopsin, which also has 7 transmembrane helices, was not useful for structure solution via molecular replacement; the arrangement of helices differs in detail, as some helices of rhodopsin include kinks, for example. The structure showed a cluster of key residues whose interactions strongly influence the color absorption properties of the 11-*cis*-retinal chromophore. The structure further revealed a set of residues involved in a possible structural change for G-protein activation, which may be extremely useful for modeling interactions in other G-Protein Coupled Receptors involved in a broad variety of biological phenomena.

The afternoon session, chaired by Prof. Chris Jacobsen of SUNY at Stony Brook, focused more on new or evolving techniques that could be of particular relevance to future X-ray FELs or PERLs. David Shapiro



Organizers and speakers of the Frontiers in Structural Biology at High-Brightness X-Ray Sources workshop (l to r): Michael Becker, Lonny Berman, Ron Stenkamp, Neali Armstrong, Rod MacKinnon, Jianwei Miao, Ben Ocko, David Shapiro, Peter Orth, and Katjuša Brejc.

of SUNY at Stony Brook, working with Dr. David Sayre, started off by giving a brief history of Sayre's work on the phasing and structure determination of single particles that started in the 1980s. He then went on to discuss progress in structure determination of a single frozen, hydrated yeast cell in the soft X-ray region at beamline X1A of the NSLS. Details were presented on sampling the continuous diffraction pattern at a spacing finer than the Nyquist interval, which provides phasing information via oversampling of the amplitude data.

This was followed by a talk of Dr. Jianwei (John) Miao of SSRL, a former student of David Sayre, who generated a great deal of excitement among the attendees by setting a goalpost for future structural work on single particles. He presented the results of recent calculations on phasing a single protein molecule via single-particle diffraction in the hard X-ray region from an X-ray FEL, such as the Linac Coherent Light Source proposed for development at Stanford. By making the optimistic assumptions of having no damage as a result of using 10-fs pulses, and by statistically averaging thousands of diffraction images of assumed known protein orientations, he showed beautiful electron density calculated with such techniques. Although the conditions of these assumptions may prove extremely challenging to realize in future practice, it is a refreshing approach and represents an attractive beacon on the horizon.

Dr. Ben Ocko of the Physics Dept. at BNL gave a helpful tutorial on X-ray diffraction from 2-dimensional/monolayer systems. Ben talked about reflectivity and grazing-incidence methods, pointing out the difficulties of obtaining atomic-resolution data with current X-ray sources and methods. He cited examples from his own work at the NSLS and from the literature on several biological and non-biological systems.

Prof. Mark Chance of the Albert Einstein College of Medicine added a unique contribution to the session, describing his group's work at the NSLS on RNA, DNA, and protein footprinting, facilitated by X-ray radiolysis of water to provide hydroxyl radicals, combined with rapid-mixing techniques. This creative combination of techniques provides a current time-resolved window in the 20-ms range. Mark cited a broad range of experiments conducted by his group using such techniques, including detailed work on RNA folding, protein-DNA interactions, and protein-protein interactions. The application of future X-ray sources for extension of such techniques, or for formulation of related but tangential techniques, will be exciting to witness.

Finally, to conclude the day, Neali Armstrong, of the laboratory of Prof. Eric Gouaux at Columbia University, presented an impressive range of structures of the water-soluble, ligand-binding core of the glutamate receptor, which functions in ligand-gated opening of transmembrane ion channels. Structures of this construct were determined with various ligands, as well as in the apo form, at beamline X4A of the NSLS. Based on the results of these structural and functional studies, a model was presented where agonists of increasing strength were correlated with increasing domain closure, thereby providing a partial mechanism for gating transmembrane channels. Also, crystal-packing interactions suggested how subunit-subunit interactions of the protein might influence allosteric modulation of activity. These studies provide critical insight for advanced study of a range of diseases.

In summary, the day's workshop generated stimulating and congenial interactions among speakers and attendees of a high level that is rarely found. It will be exciting indeed to see what new scientific gems this group of excellent scientists, and budding ones in attendance, will uncover in the future.